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REVIEW

The evolving role of liver sinusoidal endothelial cells in liver health and disease

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Abstract

LSECs are a unique population of endothelial cells within the liver and are recognized as key regulators of liver homeostasis. LSECs also play a key role in liver disease, as dysregulation of their quiescent phenotype promotes pathological processes within the liver including inflammation, microvascular thrombosis, fibrosis, and portal hypertension. Recent technical advances in single-cell analysis have characterized distinct subpopulations of the LSECs themselves with a high resolution and defined their gene expression profile and phenotype, broadening our understanding of their mechanistic role in liver biology. This article will review 4 broad advances in our understanding of LSEC biology in general: (1) LSEC heterogeneity, (2) LSEC aging and senescence, (3) LSEC role in liver regeneration, and (4) LSEC role in liver inflammation and will then review the role of LSECs in various liver pathologies including fibrosis, DILI, alcohol-associated liver disease, NASH, viral hepatitis, liver transplant rejection, and ischemia reperfusion injury. The review will conclude with a discussion of gaps in knowledge and areas for future research.

Abbreviations: AAV, adeno-associated virus; ADH1, alcohol dehydrogenase 1; AH, alcohol-associated hepatitis; ALD, alcohol-associated liver disease; ALDH, aldehyde dehydrogenase; AMPK, adenosine monophosphate-activated protein kinase; Angpt2, angiopoietin-2; AP-1, activating protein-1; TXA2, thromboxane A2; BRD4, bromodomain containing 4; CCL2, C-C motif chemokine ligand 2; CCl_4, carbon tetrachloride; CD62L, CD62 ligand; CLEC4G, C-Type Lectin Domain Family 4 Member G; c-MAF, musculoaponeurotic fibrosarcoma; CXCL1, C-X-C chemokine ligand 1; CXCR, C-X-C chemokine receptor; CYP2E1, cytochrome P450 2E1; DARC, Duffy Antigen Receptor for Chemokines; DILI, drug-induced liver injury; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; EVs, extracellular vesicles; Fcgr2b, Fc gamma-receptor IIb2; GATA4, GATA motif-binding protein 4; GIMAP5, GTPase, IMAP Family Member 5; GIT1, G-protein–coupled receptor kinase-interacting protein-1; GRK2, G-protein–coupled receptor kinase-2; HDAC6, histone deacetylase 6; HIF1α, hypoxia inducible factor 1 alpha; Hsp90, heat shock protein 90; ICAM1, intercellular adhesion molecule 1; ID1, DNA binding 1; iNOS, inducible nitric oxide synthase; ITG, integrin; IRI, ischemia reperfusion injury; Klf/KLF, Kruppel-like factor; LECT2, leukocyte cell-derived chemotaxin 2; LKB1, liver kinase B1; LPS, lipopolysaccharide; LSCEs, liver sinusoidal endothelial cells; Lyve1, lymphatic vessel endothelial receptor 1; MAdCAM-1, mucosal addressin cell adhesion molecule 1; MCD, methionine choline–deficient diet; MHC-1, major histocompatibility complex class I; MHC-II, major histocompatibility complex class II; MMP-9, matrix metallopeptidase-9; Mrc1, mannose receptor 1; MYC, myelocytomatosis oncogene; NASH, nonalcoholic steatohepatitis; NO, nitric oxide; NOD1, nucleotide-binding oligomerization domain 1; p300, histone acetyltransferase protein 300; PDGF, platelet-derived growth factor; PHx, partial hepatectomy; PLVAP, plasmalemma vesicle-associated protein; PPAR, peroxisome proliferator activated receptor; ROS, reactive oxygen species; S1PR1, sphingosine-1-phosphate receptor 1; SAMe, S-adenosylmethionine;scRNA-seq, single-cell RNA sequencing; shRNA, short hairpin RNA; SK1, sphingosine kinase 1; TCR, T-cell receptor; TG, triglyceride; Th1, T helper 1; Tie1, tyrosine kinase with Ig-like and EGF-like domains 1; TLR9, toll-like receptor 9; TRAIL, TNF-related apoptosis-inducing ligand; VAP-1, vascular adhesion protein-1; VCAM1, vascular cell adhesion molecule 1; VEGFR-2, vascular endothelial growth factor receptor-2; vWF, von Willebrand factor; Wnt2, wingless-related integration site 2.

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INTRODUCTION

LSECs are unique to the liver and comprise a distinct subpopulation of endothelial cells (ECs) even within the liver itself. The distinguishing features of LSECs have traditionally been defined by overt structural differences from other endothelia, such as lack of a basement membrane and the presence of fenestration. Rapid advances in techniques including single-cell analysis, however, have led to a broader understanding of LSEC heterogeneity even within their unique phenotype, and have refined our understanding of this singular population at a molecular level. This enhanced understanding of the distinct molecular biology of LSECs has further elucidated their roles in multiple conditions in health and disease and has led to an increased appreciation of LSEC endotheliopathy (dysfunction, increased adhesion molecule expression, and cytokine and chemokine secretion) in liver pathology. In this article, we will give an overview of advances in defining LSEC biology including resolution of LSEC heterogeneity, LSECs in aging and senescence, and the role of LSECs in liver inflammation and regeneration. We will then review how these advances inform our current understanding of the role of LSECs in liver fibrosis, DILI, alcohol-associated liver disease, nonalcoholic liver disease, liver transplant rejection, and ischemia reperfusion injury (IRI) (Table 1). We will conclude with a discussion of areas for further research in LSEC biology. We will not discuss the role of LSECs in HCC, as this has been recently reviewed.[33,34] Liver diseases with more limited data such as vascular liver disease, noncirrhotic portal hypertension, and cholestasis are outside the scope of this review.

LSEC heterogeneity: normal versus cirrhosis

LSECs are the major EC type in the liver, accounting for nearly 90% of the total liver EC population.[35] Development of single-cell sequencing technology has enabled us to identify heterogeneity within the LSEC population along with their corresponding spatial distributions, allowing us to link zonal LSEC populations to their functions in both normal and pathological conditions. An overview of this LSEC zonation is presented in Figure 1.

A study by MacParland et al.[38] revealed transcriptomic profiles of heterogeneous hepatic EC populations from healthy human donor livers using scRNA-seq technology and determined 3 EC populations, including zone1 LSECs, zone 2/3 LSECs, and vascular ECs. In this study, zone 1 LSECs represent the periportal LSECs, which show enriched expression of F8 and PECAM1, with little expression of CD32b, Lyve1, stabilin-2 (Stab2), and CD14. Zone 2/3 LSECs are considered to be central venous in origin because of their enriched expression of CD32b, Lyve1, Stab2, and low expression of vWF. The cluster of vascular ECs was characterized by low or no expression of LSEC markers, such as Lyve1, Stab2, and CD32b. Another study led by Itzkovitz demonstrated the zonation patterns of liver EC genes in normal mouse livers by the paired-cell RNA sequencing approach, which profiled the gene expression of hepatocytes and loosely attached adjacent LSECs and determined localization of the ECs in liver lobules based on the expression of well-defined hepatocyte zonal landmark genes.[39]

Recently, Su et al.[35] determined spatial/zonal characteristics of LSECs in normal and cirrhotic mouse livers and identified the transcriptomic changes in these zones associated with liver cirrhosis. Furthermore, they determined the relationships between these transcriptomic changes and the phenotypic changes observed in liver cirrhosis, such as capillarization, endocytic capacity, and vascular tone. Capillarization of LSECs is characterized by a change in their phenotype to resemble common vascular ECs more closely, specifically loss of fenestrae and formation of a basement membrane. These changes are thought to cause activation of HSCs, and thereby liver fibrosis/cirrhosis progression.[40,41] The study by Su et al[35] demonstrated that capillarization was most severe in zone 3 LSECs, suggesting that pericentral LSECs are most vulnerable in the microenvironment of cirrhotic livers. Comparison of gene expression related to LSEC capillarization between control and cirrhotic livers revealed downregulation of typical LSEC genes such as lymphatic vessel endothelial receptor 1 (Lyve1), Cd32b, and Flt4 in cirrhotic mice, mostly in zone 3. Furthermore, there was a significant upregulation of CD34 in all zones of LSECs of cirrhotic mice (6.3-fold in average), with the highest expression of CD34 around zone 3 in cirrhotic liver compared with other zones. However, the mechanism of LSEC capillarization is still not well understood. VEGF produced by hepatocytes and HSCs maintains the phenotype of LSECs.[42] However, VEGF secretion is increased in cirrhotic livers,[43] suggesting that capillarization of LSECs may be related to impairment of downstream signaling of VEGF rather than lack of VEGF per se. Furthermore, in mouse cirrhotic liver, both VEGF receptor kinase insert domain receptor and coreceptor neuropilin-1 are most downregulated in zone 3 LSECs, which may contribute to LSEC capillarization in this zone 3.[35]

LSECs are known as one of the most effective scavengers in the body because they clear wastes and pathogens originating from the gut and the systemic circulation.[44–46] This activity is because of their expression of various endocytosis receptor genes including scavenger receptors (Scarb1, Scarb2, Stab1, and Stab2)[46] and mannose receptor 1 (Mrc1)[47] in addition to the genes of related activities such as Fc gamma-receptor IIb2 (Fcgr2b/CD32b).[48] All these genes are downregulated in LSECs from a mouse model of cirrhosis, with the most prominent

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Table 1 Role of LSEC in liver pathobiology and potential therapeutic interventions

Disease/condition	LSEC-mediated mechanisms	Potential therapeutic interventions	References
Senescence	Reduced LSEC fenestrae and profibrogenic phenotype	Simvastatatin through the activation of Sirtuin 1-associated pathway reduces LSEC dysfunction and profibrotic pathway in rats with cirrhosis	[1–3]
Partial hepatectomy	LSEC-derived NO promotes hepatocyte proliferation	L-arginine increases LSEC NO production, which downregulates SAME and promotes hepayocyte proliferation in rat	[4]
Liver fibrosis, portal hypertention, and prothrombotic endotheliopathy	LECT2/Tie1 signaling enhances liver fibrosis via dysregulated portal angiogenesis and sinusoidal capillarization	The adeno-associated virus serotype 9 (AAV9) with a short hairpin RNA (shRNA) of LECT2 (AAV9-LECT2-shRNA) together with VEGF neutralizing antibody ameliorated CCl4-induced liver fibrosis in mice	[5]
	LSEC KLF2 is downregulated in cirrhosis. KLF2 possesses vasoprotective and antifibrotic effect, it increases the expression of antithrombotic factors and abrogate the thrombotic endotheliopathy in fibrosis	Simvastatin via promoting KLF2 induction improves fibrosis and portal hypertention	[6,7]
	Capillarized LSEC deposit extracellular matrix in the space of Disse	eNOS activator reverses LSEC mesenchymal features and fibrogensesis in mice	[8]
	Increased CXCL1 release by LSEC culminates in neutrophil extracelular traps formation and microvascular thrombosis	In mouse models, genetic deletion of neutrophils elastase impairs thrombosis, fibrosis, and ameliorates portal hypertention	[9]
		The anticoagulant rivaroxaban improves NO bioavailability and endothelial dysfunction	[10]
Alcohol-associated liver disease	Chronic induction of LSEC CYP2E1 and Hsp90 acetylation decreases HSP90 its interaction with eNOS and reduces NO production	Overexpression of histone deacetylase 6 (HDAC6) rescue HSP90 acetylation in LSECs and NO production and endothelial dysfunction	[11]
	Increased LSEC chemokine production and neutrophils recruitment to the liver	CXCL1 neutralizing antobody	[12]
DILI	Acetaminophen-induced injury: vWF accumulation enhances hepatic platelet retention and impairs liver repair	Blocking vWF or the platelet integrin $\alpha_{IIb}\beta_3$ reduces hepatic platelet aggregation and accelerates liver repair	[13]
	Acetaminophen-induced injury: Podaplanin released by macrophage binds to the platelet cognate receptor CLEC-2, enhance platelets sequestration and worsens liver injury	Blocking platelet CLEC-2 signaling enhances liver recovery by increasing TNF-α production and reparative hepatic neutrophil recruitment	[14]
	Chemotherapeutic agents and herbal medicines-induced toxic injury: sloughing of the sinusoidal endothelium, infiltration of blood components into the space of Disse, and procoagulant changes all lead to the sinusoidal obstruction syndrome	Prophylaxis: low-dose unfractionated heparin and ursodiol	[15,16]
		Treatement: defibrotide	[17]
Nonalcoholic fatty liver disease	Reduced NO bioavailability secondary to insulin resistance and Notch activation culminate in NO-ET-1 inbalance and LSEC dysfunction	Insulin sensitizers, Notch inhibitors, eNOS activator, and endothelin receptor antagonists improve LSEC dysfunction	[18–20]
	Increased LSEC adhesion molecule expression and proinflmmatory leukocyte adhesion promote liver inflammation	Targeting the LSEC adhesion molecule (VCAM1 or VAP-1) or the leukocyte binding partner (ITGβ1, ITGα4, ITGα4β7, or L-selectin) is beneficial in mouse models of NASH	[21–26]

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Table 1. (*continued*)

Disease/condition	LSEC-mediated mechanisms	Potential therapeutic interventions	References
Ischemia repefusion Injury	Hypoxia-induced ATP depletion	Normothermic machine perfusion minimizes anaerobic metabolism, and restores ATP	[27,28]
	Increased reactive oxygen species (ROS) generation	Recombinant form of the antioxidant human manganese superoxide dismutase added to the cold storage solution prevents oxidative stress, and reduces vWF, and ICAM1 expression in rat and human	[29]
	Lack of hemodynamic stimulation of shear stress reduced KLF2 expression impairs NO production	Simvastatin added to the cold preservation solution restores KLF2 in a rat experimental model	[30]
	Increased LSEC adhesion molecule expression, cytokine expression, and proinflammatory myeloid cell infiltration	The S1PR1 agonist increases LSEC eNOS expression, and decrease VCAM1 and proinflammatory cytokines expression, reducing myeloid cell infiltration and liver injury	[31]
	Impaired regeneration	Liver-selective metallopeptidase-9 (MMP-9) inhibition in the rat accelerates liver regeneration by preventing proteolytic cleavage of hepatic VEGF	[32]

Abbreviations: CLEC4G, C-Type Lectin Domain Family 4 Member G.eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; ICAM1, intercellular adhesion molecule 1; ITG, integrin; KLF, Kruppel-like factor; NO, nitric oxide; SAMe, S-adenosylmethionine; VAP-1, vascular adhesion protein-1; VCAM1, vascular cell adhesion molecule 1; vWF, von Willebrand factor.

downregulation in zone 3 LSECs, suggesting decreased endocytic and clearance capacities of these cells.[35] This may make cirrhotic patients more vulnerable to infection and systemic inflammation. The decreased endocytic capacity of LSECs is an indicator of their capillarization. Hence, decreased CD32b expression is also used as a marker of LSEC capillarization in some experimental in vivo studies.[49]

In cirrhotic mouse livers, genes known to promote endothelial nitric oxide (NO) synthase (eNOS) expression are similarly downregulated in all the zones, indicating the dysfunction of vascular tone throughout the sinusoidal microcirculation.[35] LSECs respond to increased shear stress to maintain normal vascular tone by promoting NO production by eNOS.[50] The loss of this property is one of the representative features of endothelial dysfunction in cirrhosis.[43,51] Some transcription factors, such as the Kruppel-like factors (Klf2 and Klf4) and activating protein-1 (AP-1), are induced by shear stress and are responsible for increased eNOS expression and activity.[52,53] Both Klf2 and Klf4 were downregulated in LSECs of cirrhotic mice.[35] Similarly, some of the major AP-1 components, such as Fos, Fosb, Jun, and Junb were remarkably suppressed in LSECs of cirrhotic livers as well. Collectively, these findings suggest that LSEC dysfunction and increased vascular tone throughout the sinusoidal microcirculation of cirrhotic livers contribute to the development of portal hypertension.

A study from the Henderson laboratory performed extensive scRNA-seq analyses of all liver nonparenchymal cells, including liver ECs, isolated from human cirrhotic livers explants. They identified 2 disease-specific EC populations, CD34+ plasmalemma vesicle-associated protein (PLVAP$^+$)VWA1$^+$ and CD34 + PLVAP$^+$ACKR1$^+$ ECs.[54] The authors named them "scar-associated ECs" but did not demonstrate their origins. Similarly, a significant upregulation of CD34, PLVAP, and ACKR1 in LSECs of all zones was observed in cirrhotic mouse livers,[35] suggesting that the disease-specific EC populations found in human cirrhotic livers may be derived from LSECs, whose gene expression profiles are altered in liver cirrhosis. An independent study by the Henderson group showed that the zonation pattern of HSCs was conserved between healthy and fibrotic mouse livers and that pericentral HSCs were predominant pathogenic collagen-producing HSCs in liver fibrosis,[55] raising the interesting possibility of pathogenic cross talk between the pericentral HSCs and the dysfunctional zone 3 LSECs susceptible to capillarization in cirrhotic livers, as identified by Su et al.[35]

The role of LSEC in the hepatic microvascular inflammatory response

Under basal conditions, LSECs have low expression of the leukocyte rolling mediators E-selectin and P-selectin, hence leukocytes adhesion to LSECs occurs independent of rolling in the liver sinusoids.[56,57] In addition to the classical adhesion molecules, LSECs express atypical adhesion molecules such as vascular adhesion protein-1 (VAP-1) and scavenger receptors

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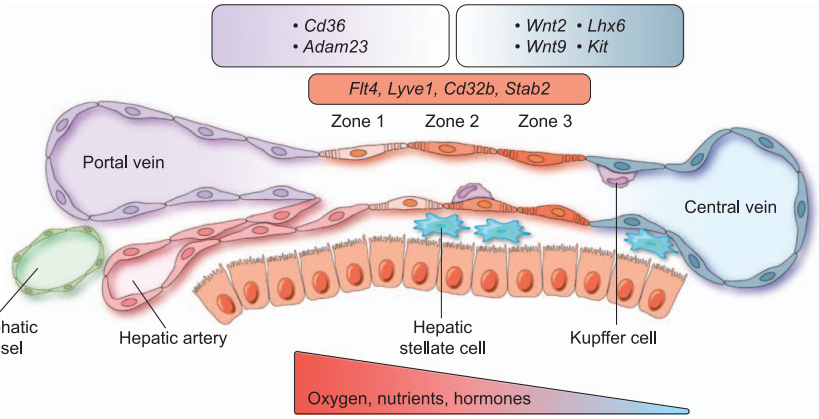

FIGURE 1 Heterogeneity of liver endothelial cell population. Liver consists of various types of endothelial cells (ECs), including LSECs with zonal differences, portal and central venous, hepatic arterial, and lymphatic ECs. LSECs account for nearly 90% of the total liver EC population, being the major liver ECs. Flt4, Lyve1, Cd32b, and Stab2 are highly expressed by LSECs. CD36 and Adam23 are expressed highly around periportal zone 1 LSECs and their expression diminishes toward zones 2 and 3. On the other hand, Wnt2, Wnt9, Lhx6, and Kit are abundantly expressed by central venous ECs and pericentral zone 3 LSECs. Similarly, their expression diminishes toward the zones 2 and 1.[35–37]

like Stabilin 1 and 2 (Stab 1 and 2).[58] VAP-1 is a membrane bound amine oxidase, which acts as an inducible ectoenzyme during inflammation. The trafficking of immune cells to the inflamed liver is tightly regulated by chemokines signaling and precise binding interactions between the Ig-like cell adhesion molecules including intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), mucosal addressin cell adhesion molecule 1 on the LSEC surface and specific integrin (ITG) binding partners on the leukocyte.[58] Firm adhesion is mediated by various subsets of leukocyte ITGs, for example, neutrophil ITGαMβ2 (or Mac-1), ITGαLβ2 or [lymphocyte function-associated-1], ITGαxβ2, and ITGαdβ2 bind to ICAM1 on LSECs, whereas ITGα4β1 (VLA-4) and ITGα4β7 (LPAM-1) expressed on most immune cells bind mainly to VCAM1 and mucosal addressin cell adhesion molecule 1 on LSECs. Chemokine signaling differentially phosphorylates the ITGβ tail regulating the leukocyte ITG binding affinity to its endothelial ligand promoting firm adhesion.[59] Stable adhesion is followed by the transendothelial migration of the immune cells to the inflammatory focus in the liver parenchyma.[60] Transendothelial migration occurs either through the paracellular route, or through the LSEC body by "transcellular crawling" mediated by the rearrangement of clustered ICAM1 around the adherent leukocyte, and the subsequent development of a transcellular channel.[61] Transendothelial migration is considered the point of no return in the inflammatory response and requires 3 key elements including optimal concentration of chemokines (chemotaxis), density of adhesion molecules (haptotaxis), and cellular stiffness (durotaxis) modulated by LSEC cytoskeletal rearrangements.[62] All these elements are potentially amenable to therapeutic interventions to abrogate the sterile inflammatory response in the liver.

LSEC modulation of adaptive immunity

The key tolerogenic phenotype of LSECs as Ag-presenting cells can be altered in the situation of infection or leveraged in a protective manner to combat autoimmunity. LSECs play an important role in the recruitment and function of immune cells in the liver in viral infection, and new studies have advanced our understanding of this component of LSEC biology. In a mouse model of HBV infection, CD8+ T-cell recruitment and antiviral activity hinged on platelets attached to LSEC hyaluronan, and these platelet aggregates formed the preferred docking sites of CD8+ T cells. The highly specialized, fenestrated morphology of LSECs then allows these CD8+ T cells to extend processes through the fenestrae to survey hepatocytes for viral infection and exert their effector function.[63] Mouse models of HBV infection have provided additional insight into the stimuli that can lead LSECs away from tolerogenic T-cell priming and toward promotion of effective antiviral immunity. HBeAg has been shown to promote the LSEC enhancement of cytotoxic T-lymphocyte function through IL-27 and TNFα.[64,65] HBeAg has additionally been shown to induce a feedback loop with increased matrix metalloprotease 2 (MMP2) production by LSECs, which subsequently leads to the release of soluble CD100, a mediator able to abrogate LSEC suppression of T-cell IFNγ production, thereby enhancing antiviral activity.[66] LSECs additionally express nucleotide-binding oligomerization domain 1 (NOD1), which when stimulated is able to overcome the basal tolerogenic phenotype of LSECs and enhance IFNγ and IL-2 production by HBV-specific T cells.[67] Further work building on these results demonstrated that the promotion of antiviral T-cell responses by stimulation of NOD1 in LSECs is enhanced by natural killer cell recruitment to LSECs in a CXCR3-dependent

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manner, and that natural killer cells promote LSEC enhancement of antiviral activity of T cells in a mouse model of HBV infection.[68]

The tolerogenic properties of LSECs have yielded additional insights into inhibition of T-cell function as well. LSEC priming of CD8+ T cells in the context of LSEC-derived programmed cell death 1 ligand 1 signaling facilitated the discovery of the role of Arl4d in the induction of a T-cell phenotype deficient in IL-2 production.[69] Additional details of the tolerogenic mechanisms of LSECs have recently been described, revealing that LSECs block metabolic changes that are associated with a more immunogenic Ag-presenting phenotype in response to lipopolysaccharide in other Ag-presenting cells, and that LSEC-primed CD8 T cells develop into a unique population of memory cells through IL-6 trans-signaling and Stat3 signaling leading to upregulation of FOXO1.[70] In chronic liver disease, however, these tolerogenic properties of LSECs may be altered, as shown in an animal model of cirrhosis in which LSECs functioned as Ag-presenting cells to stimulate a proinflammatory CD4 + Th17 T-cell response.[71]

Because of their propensity for inducing tolerance, LSECs are an intriguing target for therapeutics for autoimmune disease. One recent study has tested this approach, utilizing LSEC-selective oleic acid–stabilized superparamagnetic iron oxide nanoparticles loaded with an autoantigen in a mouse model of autoimmune cholangitis. These Ag-loaded nanoparticles were successful in inducing tolerogenic cross-presentation of Ag and protecting the mice from autoimmune disease.[72] Future studies will reveal more about how to leverage both immunogenic and tolerogenic properties of LSECs to fight infection and prevent disease.

Aging and senescence

Increased appreciation of detailed LSEC phenotyping also highlights the importance of aging in LSEC biology. Aging is associated with a progressive decline in liver function and augmented incidence and severity of chronic liver diseases.[73–75] Although initial studies suggested that age does not induce major changes in LSECs,[76] more recent investigations have demonstrated substantial age-related changes in LSEC structure and phenotype that in turn affect liver function.[1,77–81] Aged LSECs become pseudocapillarized and resemble capillaries seen in systemic vascular beds because of decreased numbers of fenestrae and increased endothelial basement membrane thickness leading to impaired sinusoidal perfusion, as observed in rat, mouse, and human liver sections.[80–82] The term “pseudo” is used to distinguish these age-related changes in LSECs from more pronounced capillarization seen in chronic liver injury[74] (*see* Liver Fibrosis and Mechanosensing section). More specifically, aged LSECs display a diminished fenestrae abundance, VEGF receptor-2 (VEGFR-2) expression, NO production, and a loss of vasodilatory capacity culminating in a moderate increase in portal pressure, as demonstrated in aged rats and validated at the mRNA level in human samples.[79] Moreover, the expression of scavenger receptors, such as Stab2, is also decreased in aged LSECs, which leads to diminished endocytic capacity and impaired waste clearance in primary LSECs isolated from aged rats.[83,84] In addition, pseudocapillarization is also accompanied by senescence-associated features in mouse, rat, and human livers.[77–79]

Aging is a key driver of LSEC senescence, a cellular state with prolonged and irreversible cell cycle arrest, macromolecular damage, distinct senescence-associated secretory phenotype, and dysregulated metabolism.[85,86] In this respect, based on studies that have been conducted in mice, rats, and human livers, aging LSECs display augmented p16 expression (indicating senescence), enhanced mitochondrial oxidative stress and increased expression of inflammatory genes, such as TNFα, IL-1, and IL-6 leading to augmented leukocyte adhesion and in vivo inflammation.[77,79,87] Senescent LSECs also worsen liver injury. For example, in a model of carbon tetrachloride (CCl_4) and phenobarbital-induced cirrhosis, aged rats exhibit an accentuated reduction of LSEC fenestrations, reduced expression of vasoprotective, vasodilatory and angiocrine factors, and an enhanced increase in portal pressure compared with young ones.[1] In addition, the decrease in NO production in elderly LSECs is associated with the activation of HSCs and profibrotic pathways.[88,89] Interestingly, liver endothelial senescence, dysfunction, and the profibrotic features in aged animals can be attenuated by simvastatin, as well as by the activation of the Sirtuin 1-associated pathway.[1–3] Indeed, simvastatin administration to aged rats with cirrhosis reduced portal hypertension, endothelium-dependent vasodilatory capacity, increased LSEC fenestrae, and decreased matrix deposition.[1] Congruently, SIRT1 overexpression in CCl_4-treated rats decreased premature senescence and ameliorated hepatic sinusoidal EC senescence, likely through p53 deacetylation.[2] In addition, shear stress and Notch-induced LSEC senescence in mice was ameliorated when SIRT1 was activated by SRT1720.[3] These data suggest that statins may serve as a potential therapeutic strategy in LSEC senescence.

Regeneration

The study of liver regeneration has yielded critical information regarding the nuanced homeostatic role normal LSECs play in the liver. The mechanisms driving liver regeneration have been studied for decades. However, how the appropriate cell mass, organization, and composition are reached during the regenerative

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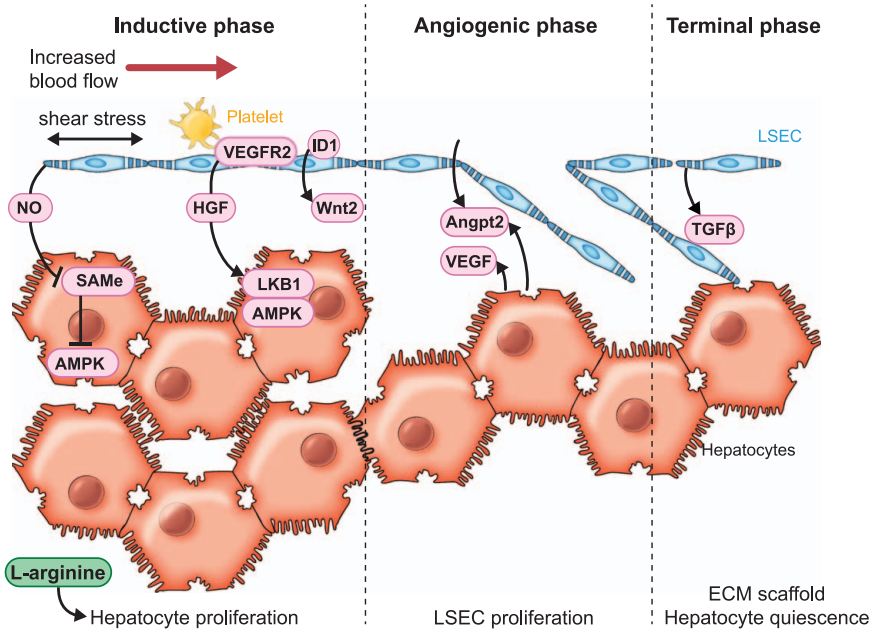

FIGURE 2 Role of LSECs during the 3 phases of liver regeneration. During the inductive phase, LSECs release NO, HGF, and Wnt2 to support hepatocyte proliferation. This is followed by the angiogenic phase where LSECs release Angpts and VEGF to support LSEC proliferation. Finally, during the terminal phase LSECs release TGFβ to support matrix remodeling and hepatocyte quiescence. Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; Angpt2, angiopoietin-2; ID1, inhibitor of the DNA binding 1; LKB1, liver kinase B1; NO, nitric oxide; SAMe, S-adenosylmethionine; TGFβ, transforming growth factor beta; VEGFR, vascular endothelial growth factor receptor, Wnt2, wingless-related integration site 2.

process remains only partially understood. Liver regeneration and regrowth to its initial mass after two thirds partial hepatectomy (PHx) is a well-organized cascade of events categorized in 3 main phases: the inductive phase where most of the hepatocytes proliferate, the angiogenic phase, and the terminal phase (Figure 2).[88,90] LSECs, which represent only 3% of the total liver volume, control hepatocyte function, and liver regeneration throughout these 3 phases.[88,90,91] After PHx and at the beginning of the inductive phase, the entire portal blood flow, normally distributed to the full-size liver, is redirected, causing a high shear stress on LSECs.[92] Although shear stress-induced LSEC senescence through Notch blunts liver regeneration,[3] shear stress activates several intracellular pathways that modulate angiocrine signaling to promote hepatocyte proliferation.[88] Shear stress induces the release of NO from LSECs,[50,93] which triggers primary isolated hepatocyte proliferation by down-regulating S-adenosylmethionine (SAMe) levels.[94,95] Complementary to these results, mice lacking glycine N-methyltransferase, which normally eliminates excess SAMe, express high levels of SAMe leading to a decrease in hepatocyte proliferation, impaired liver regeneration, and mortality.[96] Further bolstering these findings is the fact that administration of L-arginine, the substrate for NO synthase to produce NO, to rats before and after PHx enhances liver regeneration.[4] Furthermore, SAMe reduces hepatocyte proliferation through inhibiting adenosine monophosphate-activated protein kinase in mice, leading to reduced responsiveness to HGF.[97] Indeed, HGF is released by LSECs in the early hours after PHx in rats[98] and induces hepatocyte proliferation and liver regeneration through liver kinase B1 and subsequent adenosine monophosphate-activated protein kinase phosphorylation,[99–101] a process that may be triggered by the adhesion of platelets to LSECs.[102] HGF-mediated adenosine monophosphate-activated protein kinase phosphorylation increases eNOS phosphorylation leading to an increased NO release, decreased SAMe levels, and subsequent hepatocyte proliferation.[97] In addition, hepatocyte proliferation is dependent on the release of HGF and wingless-related integration site 2 (Wnt2) from LSECs downstream of the VEGFR-2/inhibitor of the DNA binding 1 (ID1) axis.[103] Indeed, hepatocyte proliferation and liver regeneration in $Id1^{-/-}$ mice with PHx are improved when mice are transplanted with $Id1^{-/-}Wnt2^{+}HGF^{+}$ LSECs.[103] LSECs also initiate liver regeneration by reducing angiopoietin-2 (Angpt2) release, which leads to decreased TGFβ 1 production and subsequent hepatocyte proliferation.[104] Later, endothelial Angpt2 release progressively increases, which participates in the switch from hepatocyte to LSEC proliferation through an autocrine mechanism and the initiation of the angiogenic phase of liver regeneration.[104] The importance of LSEC-specific Angpt2 during liver regeneration was confirmed in vivo where $Angpt2^{-/-}$ mice presented an increased hepatocyte proliferation during the initiation phase and decreased cell proliferation during the angiogenic phase,

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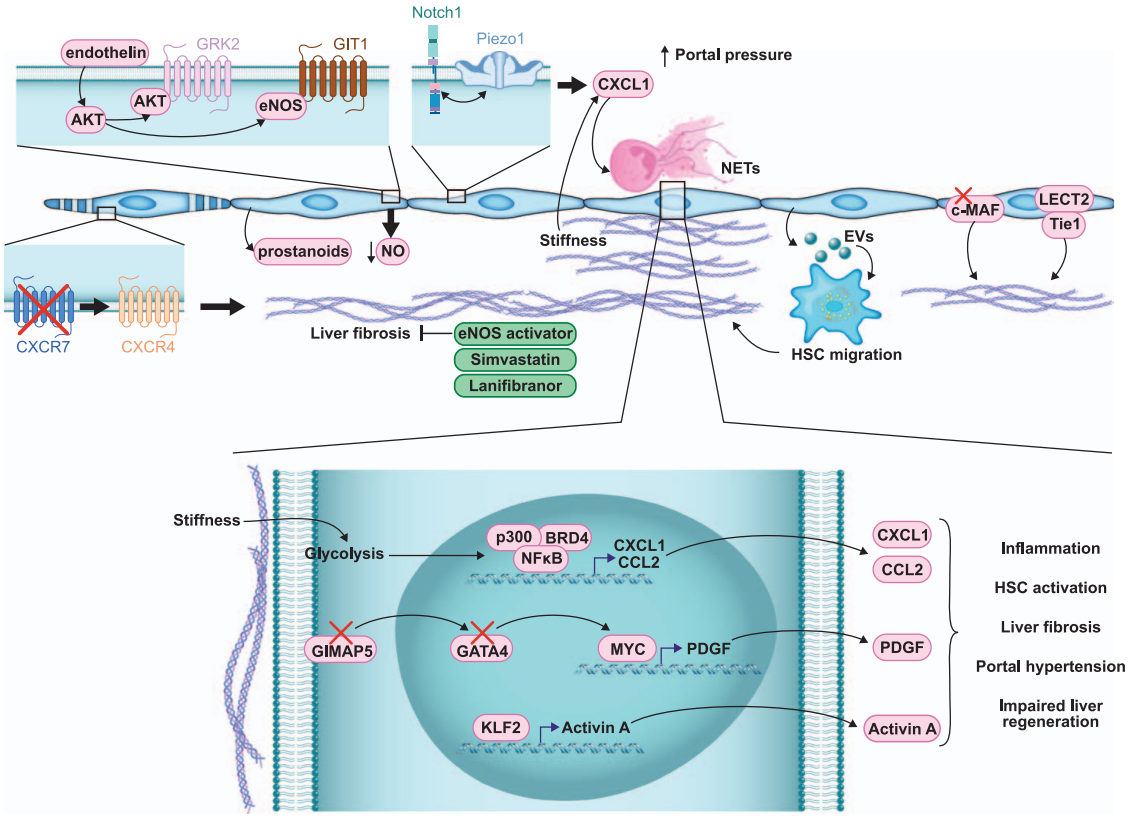

FIGURE 3 Role of LSECs during liver fibrosis. LSECs suppress C-X-C chemokine receptor 7 and favor C-X-C chemokine receptor4 expression and the release of profibrotic angiocrine signaling to promote fibrogenesis. Protein kinase B binds to G-protein–coupled receptor kinase-2 (inhibition of NO release) in addition to mediating endothelial nitric oxide synthase (eNOS) and G-protein–coupled receptor kinase-interacting protein-1 binding (activation of NO release). During fibrosis, the stiff environment increases C-X-C chemokine ligand 1 (CXCL1) release through Notch1 and Piezo1 interaction in LSECs. CXCL1 attracts neutrophils, which form neutrophil extracellular traps, thrombosis, and increase portal pressure. LSECs also release extracellular vesicles enriched with sphingosine kinase 1 to enhance HSC migration. The leukocyte cell-derived chemotaxin 2/Tie1 axis, as well as c-musculoaponeurotic fibrosarcoma repression, increase liver fibrosis through promoting the release of profibrotic signals. In a fibrotic liver, the stiffness due to matrix deposition increases glycolysis in LSECs, which promotes the expression of CXCL1 and C-C motif chemokine ligand 2 through protein 300, bromodomain containing 4, and NFκB. The repression of GTPase, IMAP Family Member 5 induces the repression of GATA motif-binding protein 4, which leads to myelocytomatosis oncogene-dependent platelet-derived growth factor expression. Kruppel-like factor 2 transcription factor promotes the expression of Activin A. These released factors promote HSC activation, liver inflammation, fibrosis, portal hypertension, and impaired liver regeneration. Finally, liver fibrosis can be abrogated by eNOS activators, simvastatin and lanifibranor. Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; Angpt2, angiopoietin-2; ECM, extravellular matrix; NO, nitric oxide; LKB1, liver kinase B1; SAMe, S-adenosylmethionine; Tie1, EGF-like domains 1; VEGFR-2, vascular endothelial growth factor receptor-2; Wnt2, wingless-related integration site 2.

as demonstrated by Ki67 immunostaining.[104] During the second angiogenic phase, hepatocytes that have already engaged in the cell cycle or just completed cell division sense relative hypoxia and secrete angiogenic factors, such as VEGF and angiopoietins, that drive LSEC proliferation, as demonstrated in several studies conducted in rats and mice.[103,105–108] Although hepatocyte proliferation peaks at 24–48 hours after PHx in mice and rats, LSEC proliferation reaches its maximum at 3–4 days after PHx.[90] Finally, LSECs play a key role during the termination phase of liver regeneration by secreting TGFβ leading to a reconstruction of the extracellular matrix scaffold and subsequent hepatocyte quiescence when examined in rats and mice.[104,109] Given the substantial contribution of LSECs in liver regeneration, modulating LSEC angiocrine signaling could be therapeutically targeted to enhance liver regeneration. Although, more studies in human liver specimens are needed to validate the human relevance of these findings.

Fibrosis and mechanosensing

In pathological conditions, dynamic changes in LSECs play a key role in facilitating liver fibrosis (Figure 3). Morphologically, in a healthy liver, LSECs are fenestrated with 3–20 fenestrations/μm^2.[110,111] The fenestrae are dynamic structures with a life span of 18 hours involving an active cytoskeleton rearrangement.[110] However, during liver injury, LSECs become capillarized by losing their fenestrations and acquiring a basement membrane

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enriched with collagen type IV and laminin.[40,110] Capillarized LSECs release vasoconstrictors such as prostanoids through cyclooxygenase-1 and exhibit diminished NO production, leading to an increase in vascular tone and portal pressure in rats.[112,113] NO production is regulated by the serine/threonine protein kinase B (PKB/AKT). In vitro experiments show that in response to endothelin, activated AKT promotes binding of eNOS to G-protein–coupled receptor kinase-interacting protein-1 (GIT1) scaffold protein, which leads to NO release (Figure 3).[114] Decreased production of eNOS-derived NO is a fundamental characteristic of LSEC dysfunction across disease etiologies,[50] and LSEC-produced NO protects the liver from fibrosis by preventing activation of HSCs.[115,116] Conversely, blocking LSEC capillarization by using an eNOS signaling activator attenuates mesenchymal features of LSECs and fibrogenesis in CCl_4-treated mice.[8]

In addition to eNOS, inducible NOS (iNOS) engages in liver fibrosis as well.[117,118] Deletion of inducible NOS in mice decreases CCl_4-mediated liver fibrosis by lowering the number of HSCs and collagen 1α1 expression.[119] Nevertheless, a conditional knockout of inducible NOS selectively in LSECs would better determine the role of endothelial inducible NOS in liver fibrosis. Although AKT can activate eNOS, AKT can also bind and deactivate the G-protein–coupled receptor kinase-2 (GRK2) to inhibit NO release (Figure 3). Moreover, GRK2-deficient mice had reduced portal hypertension as compared with controls,[120] suggesting that GRK2 participates in fibrogenesis.

A key factor to determine whether LSECs facilitate liver fibrosis or, conversely, beneficial regeneration in response to liver injury, is the balance between LSEC C-X-C chemokine receptor (CXCR) type 4 (CXCR4) and CXCR7.[121] Although the upregulation of CXCR7 leads to ID1-dependent hepatocyte proliferation and reparative liver regeneration, CXCR7 deletion promotes CXCR4 expression and subsequent liver fibrosis (Figure 3).[121] CXCR4 upregulation is associated with the release of profibrotic angiocrine signals such as TGFβ, BMP2, and PDGF-C. In line with these data, in mice with bile duct ligation liver injury, CXCR7 expression decreases while CXCR4 expression augments. In addition, liver fibrosis is improved in mice with EC-specific CXCR4 deletion.[121] The validation of these data in patient samples might be helpful to propose new therapeutic strategies.

Dysregulated angiogenesis may also promote liver fibrosis(Figure 4). For example, leukocyte cell-derived chemotaxin 2 (LECT2) binding to the endothelial tyrosine kinase with Ig-like and EGF-like domains 1 (Tie1) upregulated MAPK-dependent PPAR signaling and subsequent sinusoidal capillarization, the release of matrix proteins such as fibronectin and collagen IV, as well as liver fibrogenesis (Figure 3).[122] Furthermore, the adeno-associated virus serotype 9 (AAV9) with a short hairpin RNA (shRNA) of LECT2 (AAV9-LECT2-shRNA) or the combination of AAV9-LECT2-shRNA with bevacizumab (VEGF neutralizing antibody) ameliorated CCl_4-induced liver fibrosis in mice[5,122]

Portal hypertension is a complication of chronic liver disease, which is often characterized by high pressures in the liver sinusoids. These pressures may exert independent effects that perpetuate the LSEC pathology. LSECs undergoing mechanical stretch induced by IVC ligation in mice present an increased expression of Notch1, which interacts with the mechanosensor Piezo1, leading to the release of C-X-C chemokine ligand 1 (CXCL1) (Figure 3).[9] Then, CXCL1 attracts neutrophils to form neutrophil extracellular traps and microthrombi, which mediate portal hypertension (Figure 3).[9] Moreover, the chemokine CXCL1 is also released after mechanotransduction-induced glycolysis. In this case, in vitro stiffness-mediated glycolysis increased nuclear pore diameter and subsequent NFκB translocation to the nucleus (Figure 3).[123] In CCl_4-mediated chronic liver injury in mice, in response to TNFα, endothelial NFκB interacts with histone acetyltransferase protein 300 (p300) and bromodomain containing 4 (BRD4), leading to CXCL1 and C-C motif chemokine ligand 2 (CCL2) secretion from LSECs culminating in liver inflammation (Figure 3).[124]

In addition to NFκB, other transcription factors, such as GATA motif-binding protein 4 (GATA4) and KLF2, control angiocrine signals to promote liver fibrosis. GATA4 deficiency in LSECs leads to liver fibrosis through myelocytomatosis oncogene-dependent platelet-derived growth factor expression,[125] suggesting a protective role for endothelial GATA4. Moreover, a recent study revealed that GTPase, IMAP family member 5 (GIMAP5) upstream of GATA4 protects LSECs from capillarization. GIMAP5 deletion in LSECs results in portal hypertension, suggesting that GIMAP5 is a critical regulator of LSEC homeostasis.[126] On the contrary, activation of endothelial KLF2 leads to impaired liver regeneration through activin A expression[127] (Figure 2). However, hepatic KLF2 overexpression through adenovirus-KLF2 administration improved portal hypertension and liver endothelial dysfunction in cirrhotic rats.[6] This discrepancy regarding the role of KLF2 in liver disease might be because of the cell type–specific role of KLF2 and further studies are needed to resolve it.

Angiocrine signaling includes not only secreted soluble factors but also extracellular vesicles (EVs). Extracellular vesicles are nano-sized particles involved in cell-to-cell communication and liver disease.[128] In vitro, LSECs regulate HSC migration and signaling by releasing sphingosine kinase 1 (SK1)-enriched EVs (Figure 3). These SK1-EVs adhere to HSCs through ITG-fibronectin interaction and are internalized in a dynamin-dependent manner. In vivo, SK1 was enriched in EVs derived from sera of CCl_4-treated mice as compared with olive oil EVs.[129] In turn, HSCs also release fibrogenic EVs that are taken up by LSECs in mice.[130,131] However, further studies are needed to

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better understand HSC-LSEC cross talk through EVs during liver fibrosis.

The maintenance of sinusoidal and hepatocyte zonation is crucial for preserving liver homeostasis. As mentioned previously, multiple studies utilizing single-cell technologies implicate LSEC subpopulations in the development of liver fibrosis. A recent study reveals that musculoaponeurotic fibrosarcoma (c-MAF) expression increases gradually in LSEC subpopulations during prenatal and postnatal development and confers the sinusoidal identity of ECs. Endothelial-specific c-MAF deletion in adult mice induces the loss of the sinusoidal phenotype, increases EC proliferation, produces a mild disruption of hepatocyte zonation, and enhances CCl_4-mediated liver fibrosis (Figure 2). This study suggests that the absence of c-MAF tilts the balance in the liver toward the counterproductive healing by fibrosis.[132] As previously discussed, studies in mice and human liver biopsies demonstrate that LSEC capillarization during cirrhosis is more prominent in the pericentral subpopulation, which gain CD34 expression and decrease the expression of endocytotic receptors.[35,133]

Because of their crucial role in causing liver fibrosis, LSECs are key therapeutic target for antifibrotic studies. For example, the overexpression of KLF2 in the cirrhotic rat livers through adenovirus-Klf2 administration exerts an antifibrotic and vasoprotective effect by decreasing the portal pressure, as well as increasing eNOS expression and the response to the vasodilator acetylcholine.[6] In addition, the pan-peroxisome proliferator-activated receptor (PPAR) agonist lanifibranor leads to improved LSEC fenestrations and thus a healthy LSEC phenotype, reduced expression of the endothelial dysfunction marker von Willebrand factor, and decreased portal hypertension and hepatic fibrosis in rats with cirrhosis induced by either thioacetamide or common bile duct ligation.[134]

Endotheliopathy/thrombosis

Microvascular thrombosis has been recognized as a key player in endothelial dysfunction and portal hypertension in liver disease in recent years, and the direct relationship between LSECs and microvascular thrombosis is an area of great research interest. eNOS-derived NO serves as an inhibitor of endotheliopathy, a type of endothelial dysfunction characterized by an inflammatory and procoagulant state, by inhibiting the release of Weibel-Palade bodies, which contain P-selectin that mediates recruitment of leukocytes and thus initiates vascular inflammation.[135] Furthermore, NO can block platelet adhesion and aggregation to ECs, reducing the occurrence of microvascular thrombosis.[136] A recent study demonstrated that inhibition of coagulation utilizing the direct-acting anticoagulant rivaroxaban in animal models of cirrhosis reduced portal pressure, in part by improving endothelial dysfunction in this model as evidenced by reduced LSEC basement membrane formation and vWF expression in the rivaroxaban-treated group.[10] This suggests a direct role for microvascular thrombosis in promoting endothelial dysfunction in chronic liver disease and that inhibiting thrombosis may improve endothelial dysfunction. As discussed previously, mechanical forces exerted on LSECs in the liver sinusoids in an IVC ligation model of portal hypertension due to congestive hepatopathy directly promoted neutrophil and platelet accumulation, microvascular thrombosis, and portal hypertension, elucidating a new mechanism by which sinusoidal thrombosis contributes to worsening liver pathology.[9] However, we are still awaiting the translation of these observations to randomized clinical trials that examine the benefit of anticoagulation in patients with chronic liver disease on LSEC dysfunction and portal hypertension. Beyond a role in chronic liver disease, procoagulant endotheliopathy of LSECs characterized by increased expression of procoagulant factor VIII along with increased platelet and neutrophil adhesion is associated with acute liver injury and systemic coagulopathy in COVID-19 infection, with IL-6 transsignaling as a potential mechanism of procoagulant and proinflammatory changes in LSECs.[137]

Mechanistically, Klf2 plays a key role in preventing detrimental activation of coagulation at the level of LSECs. Klf2 increases the expression of antithrombotic factors such as thrombomodulin.[138] Statins may act directly on the aberrantly regulated KLF2 pathway to improve endothelial dysfunction in cirrhosis.[139] Recent studies demonstrated that statins ameliorate prothrombotic endotheliopathy in the liver as well. In the setting of lipopolysaccharide-induced endotheliopathy in an animal model with increased microvascular thrombosis and vWF expression by liver ECs, statin treatment upregulates KLF2 and decreases thrombosis in the liver.[140] New research has built on this approach utilizing nanotechnology that enhances simvastatin delivery to LSECs to augment KLF2.[141] This field is developing, and given that the nanotechnology in this study relied on targeting the mannose receptor on LSECs with mannan-containing nanoparticles, which while demonstrating preferential LSEC uptake lack total specificity,[142] more specialized technology should be used for further precision therapy. Further data on statins and their effect on liver endothelial health in ongoing clinical trials are eagerly awaited, with a major phase 3 clinical trial underway.[7]

In the acute setting where liver regeneration is required, such as after hepatectomy, platelet interactions with LSECs may play a beneficial role. Platelet signaling to LSECs and activation of LSEC CXCR7 has been shown to promote liver regeneration in both a chemical injury and a hepatectomy model.[143] Further studies have demonstrated signaling from platelets to LSECs, which in turn

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engage in cross talk with HSCs to promote liver regenerative signaling.[102] Taken together, these data suggest that platelets may have both beneficial and detrimental effects in the liver and highlight the complexity of microvascular thrombosis in various conditions and the need for further mechanistic studies.

Alcohol-associated liver disease

Alcohol-associated liver disease is a pressing public health issue with a near-total lack of effective pharmacologic therapy to date. The role of LSECs in this disease is increasingly appreciated, and these novel pathophysiologic pathways may hold potential for new therapeutic targets. LSEC dysfunction has been reported in alcohol-associated liver disease[144,145] and is thought to precede liver injury. A recent study revealed that LSECs could metabolize ethanol by expressing all ethanol metabolizing enzymes, including alcohol dehydrogenase 1 (ADH1) and cytochrome P450 2E1 (CYP2E1), key enzymes in alcohol metabolism that are constitutively expressed in LSECs.[11] Alcohol is metabolized first by ADH1 or CYP2E1 to acetaldehyde, a highly toxic metabolite and a known carcinogen.[146] Then, aldehyde dehydrogenase catabolizes acetaldehyde to acetate, a less active product, which can be converted to acetyl-CoA by acetyl-CoA synthetase.[146] Ethanol increases the rate of CYP2E1 gene transcription and also stabilizes CYP2E1 by preventing its degradation,[147] thereby increasing CYP2E1 levels especially in pathological conditions.[148] CYP2E1 is mainly expressed in the liver with its highest expression in hepatocytes, ~3.3-fold higher than LSECs. Whether CYP2E1 expression in LSECs is zone specific remains to be demonstrated. Given that CYP2E1 metabolizes acetaminophen, CYP2E1 in LSECs also plays a role in acetaminophen-induced LSEC dysfunction.

Chronic induction of CYP2E1 by ethanol leads to LSEC dysfunction and endotheliopathy.[11] Protein acetylation has been described in relation to ethanol exposure,[149] and acetyl-CoA, a final product of alcohol metabolism, serves as a substrate for protein acetylation.[150] In this proacetylation environment, it was determined that ethanol-driven Hsp90 acetylation and the resultant decrease of its interaction with eNOS, leading to decreased NO production, is one of the underlying mechanisms of LSEC dysfunction and liver injury under excessive alcohol consumption.[11]

Alcohol-associated hepatitis, the most severe manifestation of alcohol-associated liver disease, is associated with liver neutrophil infiltration through activated cytokine pathways and chemokine release by LSECs (Figure 4).[151–154] Among the differentially expressed genes in this disease, several CXCL chemokines such as CXCL1, 6, and 8, implicated in neutrophil recruitment, show markedly elevated expression in the livers of patients with alcohol-associated hepatitis.[151,153] Gao and colleagues reported that a high-fat diet combined with ethanol feeding elevated the production of CXCL1 in ECs as well as hepatocytes and HSCs.[12] Hence, blocking the induction of CXCL chemokines and neutrophil recruitment to the liver could be a potential therapeutic strategy for the treatment of alcohol-associated hepatitis.

Drug-induced liver injury

DILI is often attributed to hepatocyte or cholangiocyte injury. However, LSECs play a key role in this phenomenon as well. One of the most clinically important causes of DILI is acetaminophen toxicity, accounting for 2600 hospitalizations and 450 deaths from acute liver failure per year in one recent estimation.[155] LSECs have been shown to be a direct target of acetaminophen toxicity, exhibiting injury before the liver parenchyma in an animal model of acetaminophen-induced liver injury,[156] with the cytotoxicity of acetaminophen on LSECs amplified by TNF-related apoptosis-inducing ligand (TRAIL).[157] The contribution of LSECs to acetaminophen-induced liver injury is augmented by a high cholesterol diet. Free cholesterol accumulation in LSECs enhances inflammatory toll-like receptor 9 (TLR9) signaling and worsens liver injury, implicating LSECs as a key link between metabolic risk factors and acute liver failure.[158]

More recently, a key role for the endothelial-derived protein von Willebrand factor (vWF) in acetaminophen-induced liver injury has been defined in a mouse model.[13] The authors identified elevated plasma vWF levels and increased vWF accumulation in the livers of acetaminophen-injured animals, which enhanced hepatic platelet retention. Platelet retention impaired hepatic recovery, while blocking this accumulation of platelets accelerated recovery from liver injury. In addition, signaling through the platelet CLEC-2 receptor is detrimental to the liver in acetaminophen-induced injury.[14]

In addition to acetaminophen-induced liver injury, a key manifestation of toxic injury to LSECs is sinusoidal obstruction syndrome, also known as veno-occlusive disease. This syndrome results from toxic injury to the liver sinusoidal endothelium, commonly from chemotherapeutic agents and other toxins such as herbal medicines, leading to sloughing off of the endothelium and ultimately sinusoidal obstruction, manifesting clinically as hepatomegaly, jaundice, and fluid retention.[159] A major pathophysiologic event in this syndrome is the infiltration of blood components into the space of Disse after endothelial damage and subsequent dissection of ECs into the sinusoids. Platelets played a protective role in an animal model of sinusoidal obstruction syndrome by plugging the defects resulting from toxic

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injury to LSECs and preventing this dissection.[160] Although ursodeoxycholic acid is currently the preferred agent for the prevention of sinusoidal obstruction syndrome, recent studies have further implicated thrombosis in this condition by noting that low-dose heparin may be an effective prophylactic strategy by interfering with the procoagulant effects of damaged LSECs that exacerbate sinusoidal obstruction.[15,16] The key role of LSECs in the pathogenesis of sinusoidal obstruction syndrome is also highlighted by the fact that defibrotide, the only Food and Drog administration–approved therapy, functions by reducing EC activation and promoting fibrinolysis through increased expression of tissue plasminogen activator and thrombomodulin.[17]

NAFLD

NAFLD, defined by hepatic steatosis in the absence of alcohol consumption, is the most common chronic liver disease worldwide.[161] A subset of patients with NAFLD develop NASH, characterized by excess circulating toxic lipids, along with liver inflammation, and progressive liver fibrosis. Toxic lipid-induced hepatocellular stress, known as lipotoxicity, has been recognized as a driver of the inflammatory response in the NASH liver.[162] During the evolution of NAFLD, LSECs residing in a proinflammatory microenvironment under lipotoxic stress undergo various morphological and functional alterations including capillarization, dysfunction, and endotheliopathy (Figure 5).

Although preclinical studies in mouse model of high-fat diet–induced NAFLD showed preservation of fenestrae at 2, 4, 15, and 20 weeks,[163] other studies showed reduced fenestrae in high-fat high-carbohydrate diet, choline-deficient high-fat diet,[41] Choline-deficient, L-amino acid-defined[164] diet, as well as high-fat diet at 22 weeks, with preservation of fenestrae at 8 weeks. The difference observed are likely related to mouse strain used in these studies and the composition of the diet. The high-fat diet is less fibrogenic and does not recapitulate the full spectrum of the disease when compared with the other diets, including the high in fat fructose and cholesterol that showed increased capillarization markers at 24 weeks.[41] In addition, loss of fenestration in LSECs is also seen in isolated human steatosis when compared with steatohepatitis.[165] Hence, LSEC capillarization is likely to develop during the early stage of NAFLD,[164] and is partially attributed to dietary macronutrients and gut microbiota-related products.[34,166,167] However, the exact molecular mediators of LSEC capillarization in NASH are largely unknown. Capillarization of LSECs in turn promotes hepatic steatosis, because of impaired clearance of circulating triglyceride-rich chylomicron remnants, and compensatory hepatocyte de novo lipogenesis. Mice lacking LSEC fenestrations because of genetic deletion of PLVAP, an endothelial-specific membrane glycoprotein, develop severe steatohepatitis along with significant elevation of plasma LDL and triglyceride and reduction of the HDL.[168]

During the progression of hepatic steatosis, steatotic and ballooned hepatocytes exert mechanical stress on the microcirculation, which further impairs NO—endothelin-1 balance and culminates in LSEC dysfunction.[18] Increased hepatic vascular resistance in NAFLD is also attributed to impaired insulin signaling and decreased AKT-dependent eNOS phosphorylation and NO synthesis and release.[19] Hence, insulin sensitizers may attenuate LSEC dysfunction in NASH. Moreover, in rats with steatosis, increased vascular resistance is associated with enhanced liver thromboxane synthase and endothelin-1 expression and increased serum endothelin-1 level,[18] suggesting that endothelin-1 receptor blockade may ameliorate LSEC dysfunction in NASH.[169] Recently, using a mouse model of methionine choline–deficient diet-induced NASH, Fang et al.[20] showed that endothelial-specific Notch activation inhibits eNOS transcription and aggravates NASH, whereas administration of the pharmacological eNOS activator YC-1 or the Notch inhibitors DAPT and LY303947 ameliorates LSEC dysfunction and capillarization resulting in improved hepatic steatosis, inflammation, and fibrosis. Taken together, these data suggest that restoring LSECs homeostasis is beneficial in NASH.

As NASH evolves, LSECs acquire a proinflammatory phenotype characterized by increased adhesion molecule expression[21–23] that we refer to as “endotheliopathy”[170] associated with proinflammatory chemokines and cytokines release. Situated at the interface of the circulation and the liver parenchyma, LSECs participate in the host innate defense mechanism and express TLR9 in their lysosomal/endosomal compartment. Bacterial DNA, endocytosed through the LSEC scavenger receptors, activate the LSEC TLR9 leading to NF-κB signal transduction and proinflammatory cytokines (IL-1β and IL-6) release.[171] Furthermore, LSECs under lipotoxic stress or cytokine stimulation secrete C-X-C motif ligands, and the C-C motif chemokine ligands[21,58] and promote the chemotaxis of proinflammatory immune cells. In addition, LSECs through their DARC (Duffy Antigen Receptor for Chemokines) can present chemokines produced by neighboring cells to promote transendothelial migration of proinflammatory immune cells. Increased LSEC DARC expression in the fibrotic niche was identified by scRNA-seq and spatial mapping of human livers with NASH cirrhosis. In addition, functional studies confirmed that DARC enhances the transendothelial migration of leukocytes,[54] further validating the role of LSEC in shaping the liver immune cell subpopulations in NASH.

Interestingly, mice expressing a catalytically inactive form of VAP-1 or treated with a-VAP-1 neutralizing

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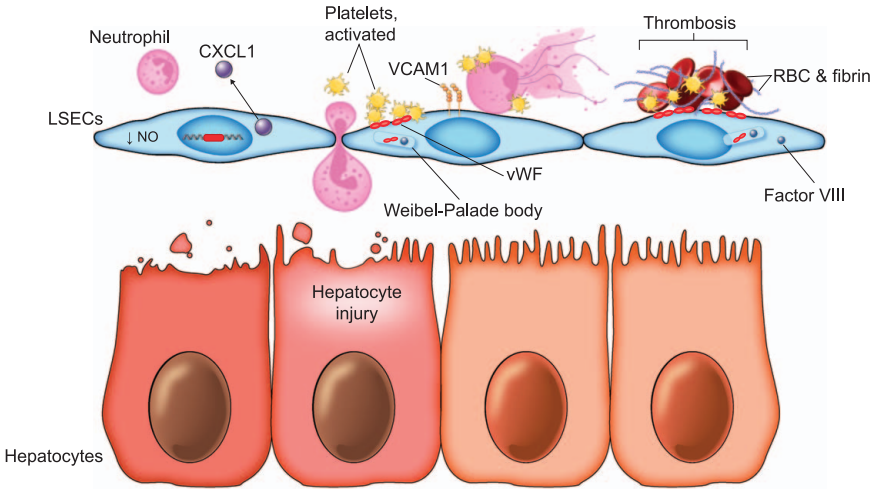

FIGURE 4 Role of LSEC in the progression of alcohol-associated hepatitis. Alcohol metabolism by CYP2E1 in LSECs leads to decreased endothelial nitric oxide synthase activity and decreased nitric oxide (NO) production by these cells in vitro and in a mouse model of alcohol-associated hepatitis (modified National Institute on Alcohol Abuse and Alcoholism model). Endothelial nitric oxide synthase-derived NO protects LSECs from endotheliopathy and abnormal platelet adhesion and aggregation to endothelial cells, reducing the occurrence of microvascular thrombosis. Chronic ethanol intake also increases expression of proinflammatory genes, such as chemokine (C-X-C motif) ligand 1 (CXCL1) and VCAM1, facilitating recruitment and adhesion of neutrophils, leading to hepatocyte injury. Decreased NO facilitates movement of vWF to the LSEC surface, which facilitates platelet attachment, an initial step of thrombus formation. Platelet-neutrophil interactions mediate neutrophil extracellular traps formation. Thrombus (blood clots) consists of accumulated platelets (platelet plug), RBCs and a mesh of cross-linked fibrin. Abbreviations: RBCs, red blood cells; VCAM1, vascular cell adhesion molecule 1; vWF, von Willebrand factor.

antibody had attenuated liver inflammation and fibrosis in methionine choline–deficient diet-induced NASH.[22] The human relevance of this observation was validated by showing significant elevation of serum levels of soluble VAP-1 in NAFLD patients.[22] Furthermore, Furuta and colleagues reported that LSEC VCAM1 expression is enhanced under lipotoxic conditions in mouse and human NASH. The authors further evaluated the therapeutic potential of targeting VCAM1 in murine NASH and showed diminished liver injury, inflammation, and fibrosis on VCAM1 pharmacological inhibition or conditional EC deletion in mice with diet-induced NASH.[23] Likewise, blocking ITGβ1 (a VCAM1 binding partner) using a neutralizing antibody also attenuated diet-induced murine NASH secondary to decreased proinflammatory monocyte adhesion and hepatic infiltration, and recovery of the restorative macrophage population.[24] Moreover, soluble VCAM1 was increased in the circulation of patients with NAFLD and correlated with advanced stages of liver fibrosis, highlighting the translational potential of these preclinical data.[172] Similarly, targeting ITGα4 (a VCAM1 ligand) reduced the proinflammatory monocyte-associated liver inflammation in mice with NASH.[21] Comparably, mice with diet-induced NASH had reduced liver inflammation, fibrosis, and metabolic dysfunction when treated with ITGα4β7 antibody, and had reduced CD4 T-cell hepatic infiltration.[25] Along the same lines, genetic deletion, or pharmacological blockade of L-selectin [also known as CD62 Ligand (CD62L)], a lymphocyte expressed ligand for ICAM1 and mucosal addressin cell adhesion molecule 1, in mice protected against diet-induced NASH. Serum levels of soluble CD62L were increased in NASH patients and CD62L hepatic expression correlated with NASH activity[26] (Figure 5). Taken together, these data suggest that LSEC luminal adhesion molecules expression is increased NASH and can be therapeutically targeted. Moreover, the soluble circulating forms of these adhesion molecules may serve as potential noninvasive biomarkers in human NASH.

Ischemia reperfusion injury

Liver ischemia reperfusion injury refers to hepatic damage on reestablishment of the hepatic circulation after the cold ischemia during organ preservation and warm ischemia period during the liver transplant surgery. Ischemia reperfusion injury is a major risk factor of early allograft dysfunction.[173] Because of the vascular nature of IRI, it is not surprising that LSECs would play a key mechanistic role. Different mechanisms of LSEC injury and dysfunction have been described in cold ischemia and warm reperfusion of the liver graft including hypoxia-induced ATP depletion, lack of hemodynamic stimulation of shear stress with flow cessation, and significant reduction of vasoprotective factors including NO through reduced expression of the shear stress-sensitive nuclear transcription factor KLF2. Low NO and high levels of thromboxane A2 (TXA2) and reactive oxygen species during IRI lead to vasoconstriction and narrowing of the sinusoid lumen, culminating in liver injury.[27]

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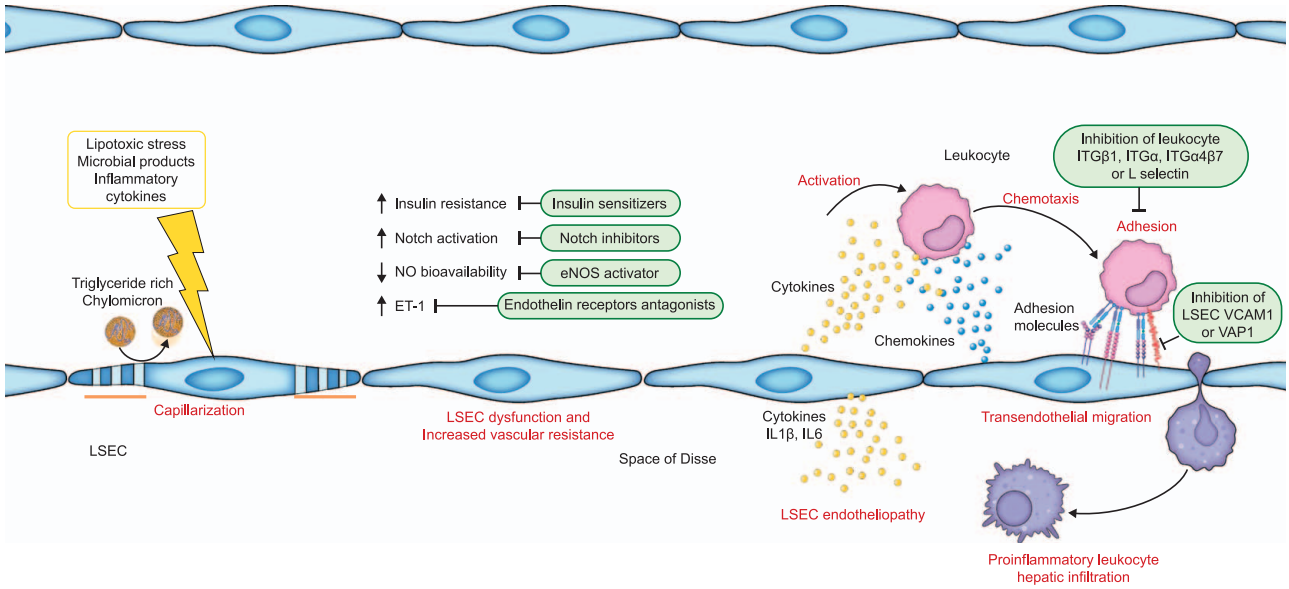

FIGURE 5 The integral role of LSEC in the progression of NASH. LSEC capillarization occurs early on during NAFLD and is partially attributed to circulating oxidized LDL-induced lipotoxic stress, and gut-derived microbial products LSEC capillarization in turn promotes liver steatosis due to decreased uptake of chylomicron remnants. Furthermore, LSEC dysfunction ensues secondary to insulin resistance, Notch activation, decreased NO bioavailability and increased ET-1. LSEC dysfunction culminates in increased vascular resistance and portal pressure. In addition, lipotoxic stress, proinflammatory cytokines and chemokines, and gut-derived microbial products promote LSEC release of proinflammatory chemokines and cytokines that chemoattract and activate circulating leukocytes. Furthermore, toxic lipid mediators and proinflammatory cytokines increase the expression of vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), mucosal address in cell adhesion molecule 1 (MadCAM-1) and the monoamine oxidase vascular adhesion protein-1 (VAP-1) on the LSEC surface. These molecules mediate proinflammatory leukocyte adhesion through engaging their integrin binding partners on the leukocyte surface, and facilitate leukocyte transendothelial migration, and hepatic infiltration. Green boxes indicate potential therapeutic interventions to abrogate NASH progression. Abbreviations. NO, nitric oxide.

LSECs undergo numerous morphological changes during IRI often manifesting as rounding and actin disassembly due to an increased intracellular calcium concentration and calpain activity.[174] In addition, LSEC injury is compounded by the reactive oxygen species and elastase produced by recruited neutrophils.[175] Activated KCs perpetuate liver injury as well by releasing proinflammatory cytokines (TNFα and IL-1β) and reactive oxygen species, which activate LSEC NF-kB, subsequently upregulating the expression of adhesion molecules such as ICAM1 facilitating neutrophil infiltration of the allograft parenchyma, as well as E-selectin and P-selectin favoring platelet adhesion and formation of vessel microthrombi. LSECs and platelets also produce platelet-activating factor, which promotes neutrophil activation and enhances reactive oxygen species generation. Interestingly, the upregulation of the LSEC costimulatory molecules CD80, and CD86 during IRI enhances their Ag presentation capability and primes the recipient immunological response for early rejection.[176]

These various pathogenic mechanisms can be abrogated by normothermic machine perfusion that maintains continuous vascular stimulation, minimizes anaerobic metabolism, and restores ATP production.[28] Likewise, multiple IRI therapeutic agents have been added to the cold storage solution or administered systemically in preclinical models to reduce reactive oxygen species generation and inflammation, while promoting regeneration. These include the recombinant form of the antioxidant human manganese superoxide dismutase, which when added to the cold storage solution, prevents oxidative stress, and reduces vWF and ICAM1 expression.[29] Likewise, adding the KLF2-inducer simvastatin to the cold preservation solution ameliorated the hepatic IRI observed on reperfusion in a rat experimental model.[30] Moreover, liver-selective metallopeptidase-9 (MMP-9) inhibition in the rat accelerates liver regeneration by preventing proteolytic cleavage of hepatic VEGF.[32] In addition, in a mouse model of IRI, the sphingosine-1-phosphate receptor 1 (S1PR1) agonist SEW287 increased LSEC AKT phosphorylation and eNOS expression, while decreasing VCAM1 and proinflammatory cytokines expression, and ultimately myeloid cell infiltration and liver injury.[31] Taken together, these data suggest that targeting the LSEC can potentially alleviate IRI and prevent graft dysfunction.

Rejection

The unique microvascular architecture of the liver enhances its tolerogenic propensity, since LSECs are strategically situated as the first line of defense and poised to engage with the immune system. LSECs

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function as semiprofessional Ag-presenting cells as they express genes involved in Ag capture and processing such as the major histocompatibility complex class II (MHC-II), which enable LSECs to present Ag to naïve CD4+ T cells. Because of their low expression of costimulatory molecules, LSECs promote the development of suppressor T cells ($CD25^{hi}$ regulatory T cells), rather than drive their differentiation to inflammatory CD4 + T cells (T helper 1 and T helper 17).[177,178] LSECs use the mannose receptor to take-up, process, and cross-present Ag through their major histocompatibility complex class I (MHC-I) receptors to CD8+ cytotoxic T cells.[179] At low Ag concentrations, this process leads to tolerance of naïve CD8+ T cells and is mediated by upregulation of the LSEC coinhibitory molecule programmed cell death 1 ligand 1, which engages its receptor programmed cell death protein-1 on naive T cells.

The function of LSECs in immunity changes, however, when Ag load is high. When the microenvironment is enriched with cytokines (IL-2 and IL-6), then Ag cross presentation to CD8+ T cells leads to a memory effector T cell differentiation, and sustained effector response likely secondary to enhanced T-cell receptor signaling that overcomes programmed cell death protein-1–mediated tolerogenic responses.[58] Likewise, LSECs may facilitate acute cellular rejection of the liver allograft as reported by Sumitran-Holgersson et al.[180] Preexisting LSEC antibodies may induce the expression of the LSEC costimulatory molecule CD86, increase the proliferation of alloreactive CD4+T cells, and downregulate the immune modulating cytokine TGFβ. It is understandable therefore that sinusoidal endotheliitis, characterized by adhesion of immune cells to LSECs, is a histopathological hallmark of acute rejection,[181] and is associated with increased expression of the LSEC Ig-superfamily of adhesion molecule ICAM1 and VCAM1,[182,183] facilitating immune cell adhesion and homing in the liver allograft. Overall, LSECs fine tune the balance and retention of immune cells within the liver and determine whether liver injury in acute rejection resolves or progresses to chronic hepatitis and end-stage liver disease. Despite their key role in liver transplant tolerance, to date, there are no antirejection therapeutic approaches that target LSECs.

Gaps in knowledge and future directions

Although our understanding of the role of LSECs in liver pathobiology and their potential as therapeutic targets has evolved over the last decade (Table 1), many fundamental gaps in knowledge persist. To date, there are no perfect markers that distinguish LSEC from other vascular ECs,[43,184] and consequently, there are no established LSEC-specific Cre mouse models.[43,185] Well-recognized LSEC markers in healthy livers include surface receptor CD32b,[186] C-Type Lectin Domain Family 4 Member G (CLEC4G),[187] Lyve1,[188] and Stab2.[189] Hence, electron microscopy remains the gold standard to identify well-differentiated LSECs by their fenestrae. Furthermore, examining specific molecular markers of dysregulated LSECs in acute or chronic experimental liver disease models poses some challenges.

Single-cell RNA sequencing analysis and single-cell proteogenomic approaches in human and mouse livers helped unravel the heterogeneous LSEC subpopulations.[35,38,39,54,187,190] In addition, an innovative approach combining transcriptomics and quantitative proteomics/phosphoproteomics in spatially sorted liver EC populations further refined the zonal-dependent vascular signaling mechanisms.[36] Guilliams et al.[191] recently combined single-cell and nuclear sequencing with spatial transcriptomic and proteomics creating a spatial proteogenomic atlas of healthy and obese human and mouse livers, this approach enabled mapping the location of different cells within the liver, and identification of interaction between neighboring cells (including LSEC and KCs) through ligands-receptors pairs. These approaches will serve as powerful tools to discover new mediators of angiocrine signaling, and paracrine interaction between LSECs and immune cells in the inflammatory focus, as well as LSECs and HSCs in perisinusoidal fibrosis. Functional studies to validate these scRNA-seq-based findings will be important to further elucidate how the changes in LSEC phenotype affect angiocrine signaling and whether these are consequences, drivers, or amplifiers of liver fibrosis and cirrhosis.

LSECs have an essential role in maintaining liver homeostasis, in part by preserving HSC and KC quiescence.[34] However, with sustained nefarious stimuli, LSECs undergo structural and functional alterations and fuel liver disease progression. Hence, promoting LSEC homeostasis is an appealing therapeutic opportunity in chronic liver diseases of different etiologies. This opportunity awaits in depth mechanistic studies to define the regulation of LSEC fenestrae dynamics and the origin and the mechanism of LSEC basement membrane deposition and extracellular matrix cross-linking during capillarization.

Although no LSEC-specific therapy exists in the field of liver transplant to date, the immunomodulatory functions of LSEC may be harnessed to develop antirejection pharmacotherapy. Furthermore, blocking the homing of immune cells by reducing the LSEC luminal adhesion molecules expression and/or function is a promising therapeutic approach in inflammatory liver diseases. The ideal LSEC-selective therapeutic agent would block the aberrant inflammatory response in the liver without compromising the host defense mechanism or the systemic immune response. Nonetheless, it is still unclear how LSECs regulate the phenotype of transmigrating immune

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cells. Similarly, the role of LSEC basement membrane in the activation of intrahepatic leukocyte subpopulations is obscure.

Although it is well recognized that LSEC dysfunction secondary to NO/endothelin-1 imbalance is a driver of increased intrahepatic resistance, the impact of LSEC cytoskeleton remodeling in response to shear stress and proinflammatory molecules on the liver microcirculatory dysfunction and the development of noncirrhotic portal hypertension is largely unknown.

Although these gaps remain, the field of LSEC biology is evolving quickly. With the rapid advances in the available methods and technology to isolate and examine LSECs using multiomics approaches, the development of complex genetic mouse models, and the interest in developing LSEC-specific pharmacotherapy, we expect that most of these gaps will begin to close soon.

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CONFLICTS OF INTEREST

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